REMARKS

Applicants thank the Examiner for her review of the instant application. Claims 1-5 remain present for examination. For the reasons stated below, the rejections of the presently pending claims are respectfully traversed.

Rejection Under 35 U.S.C. §102

The Examiner maintains the rejection of Claims 1-5 under 35 U.S.C. §102(a) as allegedly being anticipated by Barnes (WO 00/18904, published April 6, 2000). According to the Examiner, the cited reference teaches murine TANGO 215 that is 91.3% identical to SEQ ID NO:38. The Examiner asserts that the reference also teaches antibodies that bind the polypeptide.

Applicants respectfully traverse.

Applicants have argued that (1) under the doctrine established in *In re Moore* and *Stempel*, Barnes cannot serve as prior art for the present application, and (2) Barnes neither expressly nor inherently anticipates the claims.

Barnes is not Prior Art According to the Doctrine Established under *In re Moore* and *Stempel*

Applicants submit that Applicants have shown possession of as much of the claimed invention as allegedly disclosed in the cited reference prior to its publication, and, thus, under the doctrine established in *In re Moore* and *Stempel*, Barnes cannot serve as prior art for the present application. In reply to Applicants assertions regarding *In re Moore* and *Stempel*, the Examiner states:

> This is not persuasive because as of 10-26-06 the amendment to the specification changed the claim for priority to delete the relevant provisional documents. ***The priority claim to the relevant and argued provisional document has been deleted.*** As such, Applicants cannot rely upon an unclaimed priority in combination with the "Stempel Doctrine" to establish constructive reduction to practice prior to the date of the cited art. *Office Action* at page 3 (emphasis added).

This basis for maintaining the rejection is incorrect and must be removed. The Examiner's assertion is not relevant to the statutory basis on which the claims are rejected. The claims are rejected under 35 U.S.C. §102(a), which provides:

> A person shall be entitled to a patent unless —
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, **before the invention thereof by the applicant for patent**

(emphasis added)

Thus, the present statutory basis for rejecting the claims is premised on the Barnes reference allegedly describing the claimed invention **before the invention thereof by the applicant for patent**, not before application for a patent by applicant. As detailed below, Applicants have demonstrated that Barnes reference discloses no more than Applicants possessed prior to the publication of the Barnes reference. Furthermore, it is well established that an earlier filed patent application, even if not usable as a priority document under 35 U.S.C. §119, can serve as documentary evidence demonstrating prior invention. See *In re Costello*, 717 F.2d 1346, 219 USPQ 389, 392 (Fed. Cir. 1983) ("Appellants were able to reduce the invention to writing. That writing therefore constitutes documentary evidence that appellants had conceived of the invention as of the filing date."). In the present case, U.S. Provisional Application 60/096959, filed August 18, 1998, serves as documentary evidence that the Inventors possessed as much of the claimed invention as allegedly disclosed in the cited reference prior to its publication. The Examiner cannot ignore the disclosure in U.S. Provisional Application 60/096959, simply because no priority claim to the document exists. The test under 35 U.S.C. §102(a) is whether or not the claimed invention was "described in a printed publication in this or a foreign country, **before the invention thereof by the applicant for patent**." U.S. Provisional Application 60/096959, filed August 18, 1998, is documentary evidence that Applicants had possessed as much as is provided in the Barnes reference well before the publication date of the Barnes reference. Accordingly, the Examiner's refusal to consider Applicants provisional application as evidence of possession prior to the Barnes publication date is improper. Insofar as the rejection is based on this improper refusal to consider Applicants' provisional application, the rejection must be removed.

Also regarding Applicants assertions regarding *In re Moore* and *Stempel*, the Examiner additionally contends:

> Further, Applicants argue possession of the polypeptide of SEQ ID NO:38, wherein the claims and art at issue are directed to antibodies *per se*. As such, possession of SEQ ID NO:38 does not establish constructive reduction to practice or conception of the now claimed antibodies in the provisional document.

> Applicants reliance on *In re Moore* and "The Stempel Doctrine" is therefore misplaced. *Office Action* at page 3 (emphasis added).

This statement inaccurately characterizes Applicants' previous statements and incorrectly implies that the Barnes reference provides more than that which applicants possessed prior to the publication of the Barnes reference.

As previously explained, Applicants have shown possession of as much of the claimed invention as allegedly disclosed in the cited reference prior to its publication. The sequence of SEQ ID NO:38 was first disclosed in U.S. Provisional Application 60/096959, filed August 18, 1998. The invention as presently claimed was clearly possessed by Applicants at least by the filing date of the provisional application, as a reading of the entire provisional application makes clear. For example, **the provisional application discloses antibody that binds the claimed polypeptide, as well as humanized antibodies and monoclonal antibodies (see, *e.g.*, page 5, lines 8-9, page 27, line 13 through page 33, line 19, Example 7, and Claims 28-30)**. In order to assist the Examiner in confirming that which was disclosed in U.S. Provisional Application 60/096959, a copy of U.S. Provisional Application 60/096959 is enclosed herewith as Exhibit 1. Thus, the provisional application clearly demonstrates possession of the invention by Applicants by at least its August 18, 1998 filing date, which is prior to the effective date of the cited reference, April 6, 2000.

The Barnes reference discloses nothing more than that which Applicants disclosed nearly two years prior to the publication of the Barnes reference. The Examiner apparently characterizes the Barnes reference as "directed to antibodies *per se*." However, nothing of Barnes' "antibodies *per se*" extends beyond that which Applicants already possessed. Barnes' alleged disclosure of "antibodies *per se*" is a general characterization of antibodies against Barnes' "TANGO" proteins. No particular antibody against TANGO 215 is disclosed. Thus, Barnes' disclosure of "antibodies *per se*" is not a disclosure of a particular antibody against TANGO 215, but instead is a disclosure of antibodies against Barnes' "TANGO" proteins generally. As such, Barnes' disclosure of "antibodies *per se*" provides nothing more than that which Applicants disclosed nearly two years prior to the publication of the Barnes reference. Accordingly, in accordance with the doctrine established under *In re Moore* and *Stempel*, Barnes cannot serve as prior art for the present application.

The Examiner misapplies the doctrine established under *In re Moore* and *Stempel* because the Examiner requires Applicants to demonstrate possession of more than that disclosed in the Barnes reference. In contrast to the Examiner's position, the well-established "Stempel Doctrine" stands for the proposition that a patent applicant can effectively swear back of and remove a cited prior art reference by showing that he or she made that portion of the claimed invention that is disclosed in the prior art reference. (*In re Stempel*, 113 USPQ 77 (CCPA 1957)). In other words, a patent applicant need not demonstrate that he or she made the entire claimed invention in order to remove a cited prior art reference. He or she need only demonstrate prior possession of that portion of his or her claimed invention that is disclosed in the prior art reference and nothing more.

The Stempel Doctrine was extended to cases where a reference disclosed the claimed compound but failed to disclose a sufficient utility for it in *In re Moore*, 170 U.S.P.Q. 260 (CCPA 1971). More specifically, the patent applicant (Moore) claimed a specific chemical compound called PFDC. In support of a rejection of the claim under 35 U.S.C. § 102, the Examiner cited a reference which disclosed the claimed PFDC compound, but did not disclose a utility for that compound. Applicant Moore filed a declaration under 37 C.F.R. § 1.131 demonstrating that he had made the PFDC compound before the effective date of the cited prior art reference, even though he had not yet established a utility for that compound. The lower court found the 131 declaration ineffective to swear back of and remove the cited reference, reasoning that since Moore had not established a utility for the PFDC compound prior to the effective date of the cited prior art reference, he had not yet completed his "invention."

On appeal, however, the CCPA reversed the lower court decision and indicated that the 131 declaration filed by Moore was sufficient to remove the cited reference. The CCPA relied on the established Stempel Doctrine to support its decision, stating:

> An applicant need **not** be required to show [in a declaration under 37 C.F.R. § 1.131] any more acts with regard to the subject matter claimed that can be carried out by one of ordinary skill in the pertinent art following the description contained in the reference....the determination of a practical utility when one is not obvious need **not** have been accomplished prior to the date of a reference unless the reference also teaches how to use the compound it describes. (Id. at 267, emphasis added).

Thus, *In re Moore* confirms the Stempel Doctrine, holding that in order to effectively remove a cited reference with a declaration under 37 C.F.R. § 1.131, an applicant need only show that portion of his or her claimed invention that appears in the cited reference. Moreover, *In re Moore* stands for the proposition that when a cited reference discloses a claimed chemical compound either absent a utility or with a utility that is different from the one appearing in the claims at issue, a patent applicant can effectively swear back of that reference by simply showing prior possession of the claimed chemical compound. In other words, under this scenario, the patent applicant need not demonstrate that he or she had discovered a patentable utility for the claimed chemical compound prior to the effective date of the prior art reference.

While these cases discuss the ability to effectively swear back of the cited reference by way of a 131 declaration, Applicants submit that the same reasoning applies here, where Applicants' U.S. Provisional Application 60/096959, filed August 18, 1998, serves as evidence of Applicants' possession prior to publication of the cited reference. As discussed in detail above, Applicants' U:S. Provisional Application 60/096959, filed August 18, 1998, is evidence demonstrating possession of SEQ ID NO:38 and antibodies directed thereto. Barnes' disclosure provides nothing beyond which Applicants possessed. Accordingly, in accordance with the doctrine established under *In re Moore* and *Stempel*, Barnes cannot serve as prior art for the present application.

Barnes does not Expressly or Inherently Disclose Applicants' Claims

As Applicants have previously argued, even if Barnes were properly prior art, which it is not, Barnes cannot anticipate Applicants' claims because no disclosure of Barnes expressly or inherently discloses Applicants' claimed antibodies.

In reply to Applicants previous remarks, the Examiner asserts:

> [I]t is well established in the art that an antibody binds to a minimal protein epitope of 6 consecutive amino acids. The polypeptide of the prior art and the instant polypeptide set forth in SEQ ID NO:38 have 91% identity over 720 consecutive amino acids and 100% over at least 400 consecutive amino acids. Since the polypeptides would necessarily share antibody epitopes in common the antibodies of the prior art would necessarily bind the polypeptide of SEQ ID NO:38. Applicants argue Li and Lederman et al to establish that changing one amino acid can change binding specificity of a monoclonal antibody and that some antibodies may bind regions that are different. This is not persuasive

> because the claims clearly encompass monoclonal antibodies that bind the regions that are identical and the claims are not limited to or directed to the regions that are different as argued. The argument that there may be regions that distinguish the two polypeptides, does not obviate the fact that there are more regions that will not distinguish the polypeptides and the claims encompass both of these. *Office Action* at 3-4.

This argument: (1) is inconsistent with the Examiner's position in Applicants' related application that claims the polypeptide of SEQ ID NO:38; (2) mischaracterizes Applicants' statements; and (3) misapplies the doctrine of inherency. Therefore, this argument cannot serve as a basis for rejecting the claims.

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987). The pending claims recite an isolated antibody or fragment thereof that specifically binds to the polypeptide of SEQ ID NO:38. The cited reference does not expressly disclose an antibody which specifically binds to the polypeptide of SEQ ID NO:38, and the Examiner has not established that the cited reference inherently discloses an antibody that satisfies the claims. The M.P.E.P. states that:

> To establish inherency, the extrinsic evidence "must make clear that the missing descriptive matter is **necessarily** present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, **may not be established by probabilities or possibilities**. The mere fact that a certain thing **may** result from a given set of circumstances **is not sufficient**." *M.P.E.P.* §2112 ¶IV (8th ed. 2004), quoting *In re Robertson*, 169 F.3d 743, 745 (Fed. Cir. 1999) (emphasis added).

Thus, the M.P.E.P. and cited case law makes clear that only if an antibody to the proteins disclosed in the cited references necessarily specifically binds to the polypeptide of SEQ ID NO:38 can it be said to render the claimed subject matter anticipated.

Given the above standard, the Barnes reference can inherently anticipate the claimed invention **only if** the answer to the following question is "yes":

> 1. Does an antibody raised against the TANGO 215 protein of Barnes necessarily, always and without exception, possess the property of specifically binding to the polypeptide of SEQ ID NO:38?

This inquiry can be rephrased, such that Barnes inherently anticipates the claimed invention **only if** the answer to the following question is "yes":

2. Does the extrinsic evidence make it clear that there is a 100% probability that an antibody raised against the protein of Barnes possess the property of specifically binding to the polypeptide of SEQ ID NO:38?

Applicants assert that the answer to both questions clearly is "no."

From Applicants' understanding of the Examiner's previous statements, Applicants are lead to conclude that the Examiner also would answer "no" to both questions. The Examiner's description of the Barnes reference acknowledges that there are numerous difference between Barnes' TANGO 215 and Applicants' SEQ ID NO:38. The Examiner has asserted that a single amino acid substitution in a common allele can ablate binding of a monoclonal antibody. These statements lead to the conclusion that an antibody raised against the TANGO 215 protein of Barnes would not necessarily, always and without exception, possess the property of specifically binding to the polypeptide of SEQ ID NO:38.

Barnes' TANGO 215 protein differs in amino acid sequence from Applicants' SEQ ID NO:38. The Examiner confirms this by asserting that Applicants' SEQ ID NO:38 shares 91.3% sequence identity with Barnes' TANGO 215 protein. Thus, according to the Examiner, Barnes' TANGO 215 protein differs from Applicants' SEQ ID NO:38 at over 60 amino acid locations.

The extrinsic evidence demonstrates that an antibody that binds a first protein can fail to bind a second protein that differs from the first by only one amino acid. The Examiner herself has noted in Applicants' related application that claims the polypeptide of SEQ ID NO:38, "Furthermore, Lederman et al. disclose that a single amino acid substitution in a common allele ablates binding of a monoclonal antibody (see entire document) and Li et al. disclose that dissociation of immunoreactivity from other biological activities when constructing analogs (see entire document)." *Office Action dated December 26, 2006, in Appl. No. 10/063,545* at page 6. Thus, according to the Examiner's position in regard to antibodies against the polypeptide of SEQ ID NO:38, there is relevant evidence demonstrating that a single amino acid substitution in a common allele can ablate binding of a monoclonal antibody.

Since (1) Barnes' TANGO 215 protein differs from Applicants' SEQ ID NO:38 at over 60 amino acid locations, and (2) there is relevant evidence demonstrating that a single amino acid substitution in a common allele can ablate binding of a monoclonal antibody, then it follows that an antibody that binds to Barnes' TANGO 215 protein does not necessarily and always bind to Applicants' SEQ ID NO:38. The Examiner acknowledges that there are numerous difference

between Barnes' TANGO 215 and SEQ ID NO:38. The Examiner's has asserted that a single amino acid substitution in a common allele can ablate binding of a monoclonal antibody. These assertions lead to the conclusion that an antibody raised against the TANGO 215 protein of Barnes would not necessarily, always and without exception, possess the property of specifically binding to the polypeptide of SEQ ID NO:38. Thus, the extrinsic evidence makes it clear that there is not a 100% probability that an antibody raised against the protein of Barnes possess the property of specifically binding to the polypeptide of SEQ ID NO:38. In order to expedite examination and clarify issues on appeal, **Applicants request that the Examiner affirm for the record the following:**

(1) Barnes' TANGO 215 protein differs from Applicants' SEQ ID NO:38 at over 60 amino acid locations;

(2) the Examiner's own assertion in Appl. No. 10/063,545 regarding antibodies that bind SEQ ID NO:38 that "Lederman et al. disclose that a single amino acid substitution in a common allele ablates binding of a monoclonal antibody;"

(3) the Examiner's assertions under (2) would apply to the facts under (1) such that there is not a 100% probability that an antibody raised against the protein of Barnes possess the property of specifically binding to the polypeptide of SEQ ID NO:38.

Applicants have asserted no more than the Examiner has asserted: a single amino acid difference between two proteins is sufficient to destroy binding of an antibody to the protein. Applicants have relied on the Examiner's references by Lederman and Li as extrinsic evidence to support this statement, and there is no evidence of record contrary to Lederman and Li and the Examiner's characterization thereof – *i.e.*, there is no evidence of record that an antibody raised against Barnes' TANGO 215 protein will necessarily possess the property of specifically binding to the polypeptide of SEQ ID NO:38. Given the extrinsic evidence of record in the publications by Li and Lederman, and the lack of any evidence to the contrary, there exists some possibility that an antibody raised against Barnes' TANGO 215 protein will not possess the property of specifically binding to the polypeptide of SEQ ID NO:38. The Examiner apparently agrees: "Lederman et al. disclose that a single amino acid substitution in a common allele ablates binding of a monoclonal antibody" *Office Action dated December 26, 2006, in Appl. No. 10/063,545* at page 6. Accordingly, the probability that an antibody raised against Barnes' TANGO 215 protein

will possess the property of specifically binding to the polypeptide of SEQ ID NO:38 is less than 100%. Because it is less than 100%, one cannot answer the first question above in the affirmative: an antibody raised against the TANGO 215 protein of Barnes does not necessarily, always and without exception, possess the property of specifically binding the polypeptide of SEQ ID NO:38.

While it is probable, indeed likely, that an antibody to Barnes' TANGO 215 protein would bind the polypeptide of SEQ ID NO:38, it is not a certainty. This uncertainty is confirmed by the Examiner: "Lederman et al. disclose that a single amino acid substitution in a common allele ablates binding of a monoclonal antibody" *Office Action dated December 26, 2006, in Appl. No. 10/063,545* at page 6. Mere possibility, no matter how likely, is not sufficient for inherent anticipation: "Inherency, however, **may not be established by probabilities or possibilities**. The mere fact that a certain thing **may** result from a given set of circumstances **is not sufficient**." *M.P.E.P.* §2112 ¶IV (8th ed. 2004), quoting *In re Robertson*, 169 F.3d 743, 745 (Fed. Cir. 1999) (emphasis added).

The truth of the above argument is made clear by considering a hypothetical situation wherein two 500+ amino acid-containing proteins have less than 2% identity, but share a single, 100% identical 6-amino acid sequence (asserted by the Examiner as a sufficient protein epitope for antibody binding). While it is possible that an antibody raised against one protein will bind the other, this outcome is unlikely as only a fraction of the amino acids are the same. The mere fact that an antibody to the first protein ***may*** result in an antibody which binds the second protein is not sufficient for inherent anticipation: "The mere fact that a certain thing **may** result from a given set of circumstances **is not sufficient**." *M.P.E.P.* §2112 ¶IV (8th ed. 2004), quoting *In re Robertson*, 169 F.3d 743, 745 (Fed. Cir. 1999) (emphasis added). If the Examiner's argument in the present Office Action were to be applied to these facts (two proteins share a portion of their sequence that may possess the same epitope), an antibody that bound to one protein would inherently anticipate an antibody that binds to the other protein. This is not the law.

Now consider two 500+ amino acid-containing proteins where the sequence identity is 90% and the two proteins share a 100% identical 100-amino acid sequence. While it is far more likely that an antibody raised against the first protein will bind the second, this outcome is not certain as a substantial fraction of the amino acids are different. Although the probability that an

antibody against the first protein will also bind the second is greater for proteins with 90% identity and a long stretch of identical amino acids than for proteins with 2% identity and only a single identical 6-amino acid stretch, in both cases it remains a mere probability or possibility, not a certainty. There remain regions in the two proteins that can present different epitopes such that an antibody that binds to one protein does not bind to the other. For example, in some instances, it is possible that "a single amino acid substitution in a common allele ablates binding of a monoclonal antibody" *Office Action dated December 26, 2006, in Appl. No. 10/063,545* at page 6.

In both of the above cases, it remains true that an antibody to the first protein **may** possess the property of binding to the second protein. This is not sufficient: "Inherency, however, **may not be established by probabilities or possibilities**." *Id.* Thus, under the doctrine of inherency, while the facts of the two above cases vary, the law and the outcome are the same: neither case would support a rejection based on inherent anticipation.

The Examiner has made several arguments in response to Applicants' assertion that Barnes does not anticipate the pending claims. Regarding Applicants' assertion that an antibody to the TANGO 215 protein of Barnes does not necessarily possess the property of specifically binding the polypeptide of SEQ ID NO:38, the Examiner argues, "Since the polypeptides would necessarily share antibody epitopes in common the antibodies of the prior art would necessarily bind the polypeptide of SEQ ID NO:38." Restated, the Examiner's argument is that SEQ ID NO:38 and Barnes' TANGO 215 protein share sufficient sequence identity over a portion of both sequences such that an antibody that binds to one protein in that portion of the sequence must also necessarily bind to the other protein. Even if Applicants' conceded the veracity of the Examiner's argument, the Examiner's argument must fail. The Examiner has asserted that Applicants' SEQ ID NO:38 shares 91% sequence identity with Barnes' TANGO 215 protein. As Applicants' above hypothetical demonstrates, there exist regions in the two proteins that can present different epitopes such that an antibody that binds to one protein does not bind to the other. Regardless of the probability that an antibody against the Barnes' TANGO 215 protein will bind an epitope that is identically present in polypeptide of SEQ lD NO:38, there nevertheless exists a possibility that an antibody against the Barnes' TANGO 215 protein will bind an epitope that is not identically present in polypeptide of SEQ ID NO:38 such that the

antibody against the Barnes' TANGO 215 protein will not bind the polypeptide of SEQ ID NO:38. Thus, the logic of the Examiner's argument is fatally flawed because it ignores: (1) the fact that Barnes' TANGO 215 protein can possess regions that are epitopically different than that of the polypeptide of SEQ ID NO:38; (2) the possibility that an antibody against Barnes' TANGO 215 protein may bind a region that is epitopically different than that of the polypeptide of SEQ ID NO:38, and, therefore, may not bind the polypeptide of SEQ ID NO:38; and (3) the fact that inherency cannot be established when there exists the possibility of point (2). Because nothing in the Examiner's arguments rebuts the possibility that an antibody against Barnes' TANGO 215 protein may bind a region that is epitopically different than that of the polypeptide of SEQ ID NO:38, and, therefore, may not bind the polypeptide of SEQ ID NO:38, the Examiner's arguments are insufficient to sustain the inherency rejection.

In reply to the Applicants submission as evidence the publications of Li and Lederman, the Examiner argues, "This is not persuasive because the claims clearly encompass monoclonal antibodies that bind the regions that are identical and the claims are not limited to or directed to the regions that are different as argued." The Examiner's argument: (1) mischaracterizes Applicants' arguments; (2) is irrelevant to Applicants' argument that Barnes does not inherently disclose that which is claimed; and (3) is non-responsive to the evidence of record and the Examiner's own affirmation thereof.

First, Applicants have never argued that Applicants' claims are limited to or directed to only the regions that differ between Barnes' TANGO 215 protein and the polypeptide of SEQ ID NO:38. Such a statement by the Examiner serves to confuse the record as to Applicants' characterization of that which Applicants claim, and must be rescinded in order to remove this confusion.

Second, it is irrelevant to the question of inherency that Applicants are not claiming antibodies directed to the solely regions that differ between Barnes' TANGO 215 protein and the polypeptide of SEQ ID NO:38. The question of inherency is an inquiry into that which is or is not inherently disclosed in the cited reference, the question of inherency is not an inquiry into Applicants' claim scope. For the reasons stated above, an antibody to Barnes' protein does not necessarily possess the property of specifically binding the polypeptide of SEQ ID NO:38 as recited in the pending claims. Barnes does not disclose an antibody to a particular portion of

TANGO 215. Instead, Barnes generically discloses an antibody to TANGO 215, and therefore, in order to anticipate, the generically disclosed antibody must necessarily possess the claimed property. Because the extrinsic evidence establishes that it does not, it cannot inherently anticipate the pending claims.

Third, the Examiner's assertions regarding Applicants' claim scope serve no role in dismissing the teachings of Li and Lederman. The Examiner places no evidence or argument on the record to doubt the teachings of Li and Lederman. The Examiner has, in fact, affirmed these teachings in a patent application directed to the polypeptide of SEQ ID NO:38: "Lederman et al. disclose that a single amino acid substitution in a common allele ablates binding of a monoclonal antibody (see entire document) and Li et al. disclose that dissociation of immunoreactivity from other biological activities when constructing analogs (see entire document)." *Office Action dated December 26, 2006, in Appl. No. 10/063,545* at page 6. The Examiner must either present evidence or argument to doubt these teachings and her own assertions regarding these teachings, or else the Examiner must accept the truth of these teachings and her own assertions. Since the Examiner has in Appl. No. 10/063,545 confirmed the teachings of Li and Lederman, and the Examiner presently fails to place evidence or argument on the record to doubt the teachings of Li and Lederman, Applicants request that the Examiner acknowledge the truth of the teachings of Li and Lederman.

The Examiner also insists that the burden is on Applicants "to show a novel or unobvious difference between the claimed product and the product of the prior art" because the PTO does not have the facilities "for examining and comparing applicant's protein with the protein of the prior art." *Office Action* at 3. The Examiner relies on *In re Best* for this proposition. As is clear from the guidelines laid out by the court in *In re Best*, Applicants bear no such burden. The court in *In re Best* required that the PTO have a reason to believe that the cited art **inherently** possesses the characteristic relied on for novelty in the claimed subject matter. As Applicants have previously established, there exist the possibility that an antibody that binds Barnes' TANGO 215 protein does not also bind the polypeptide of SEQ ID NO:38. The Examiner herself has provided a reason to doubt that an antibody that binds Barnes' TANGO 215 protein would also bind the polypeptide of SEQ ID NO:38 ("Lederman et al. disclose that a single amino acid substitution in a common allele ablates binding of a monoclonal antibody" *Office Action*

dated December 26, 2006, in Appl. No. 10/063,545 at page 6). Therefore, the Patent Office does not have a reason to believe that the cited art inherently, *i.e.* necessarily or always, possesses the characteristic of the claimed subject matter that is not disclosed in the cited art – specifically binding to a polypeptide of SEQ ID NO:38. In the absence of a reasonable basis to believe the characteristic is inherent, the Examiner cannot rely on *In re Best* to shift the burden to the Applicants, and therefore the Examiner's reliance on *In re Best* is misplaced.

Even if the Examiner had a reasonable basis to believe that the characteristic is necessarily and always present in the cited reference, which she does not, *In re Best* merely states that the PTO may "require the applicant to prove that the subject matter shown to be in the prior art does not possess the characteristic relied on." It is well-established that the evidentiary standard to be used throughout an *ex parte* examination in setting forth a rejection is a preponderance of the evidence, or "more likely than not" standard. *In re Oetiker*, 977 F.2d 1443, 1445, 24 U.S.P.Q.2d 1443, 1444 (Fed. Cir. 1992). Therefore, even if the Examiner is justified in shifting the burden of proof to the Applicants to rebut the inherency argument, Applicants need only show that it is more likely than not that an antibody to Barnes' TANGO 215 protein does not necessarily, *i.e.* always, specifically bind to the polypeptide of SEQ ID NO:38. Applicants have provided uncontested evidence which establishes an antibody to Barnes' TANGO 215 protein does not inherently possess the property of specifically binding to polypeptide of SEQ ID NO:38. The Examiner's own statements confirm this uncontested evidence ("Lederman et al. disclose that a single amino acid substitution in a common allele ablates binding of a monoclonal antibody" *Office Action dated December 26, 2006, in Appl. No. 10/063,545* at page 6). Thus, Applicants have provided unrebutted evidence which establishes that it is more likely than not that the claimed features are not inherent in Barnes, since "Lederman et al. disclose that a single amino acid substitution in a common allele ablates binding of a monoclonal antibody" *Office Action dated December 26, 2006, in Appl. No. 10/063,545* at page 6.

In conclusion, the evidence of record makes clear that Applicants' claims are novel over Barnes because Barnes is not prior art to Applicants' claims and because Barnes does not expressly or inherently disclose Applicants' claimed invention. The Examiner provides no basis

to conclude otherwise. In view of the above, Applicants respectfully request removal of this rejection of the claims.

CONCLUSION

In view of the above, Applicants respectfully maintain that claims are patentable and request that they be passed to issue. Applicants invite the Examiner to call the undersigned if any remaining issues may be resolved by telephone.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: July 18, 2007

By: _____
AnneMarie Kaiser
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Attorney of Record
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071607

Exhibit 1

PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c)

"EXPRESS MAIL" MAILING LABEL

Number: EL157029199US Date of Deposit: August 18, 1998

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Typed or Printed Name: Kevin Campbell

Signed: [signature]

Docket Number:	P-66818/WHD/MTK (G-tech Docket: PR1588)	Type a plus sign (+) inside this box →	+

INVENTOR(s)/APPLICANT(s)

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
WOOD	WILLIAM	I.	San Mateo, California

TITLE OF THE INVENTION (*280 characters max*)

NOVEL POLYPEPTIDES HAVING SEQUENCE SIMILARITY TO FACTOR C AND NUCLEIC ACIDS ENCODING THE SAME

CORRESPONDENCE ADDRESS

Walter H. Dreger, Registration No. 24,190
FLEHR HOHBACH TEST ALBRITTON & HERBERT LLP, 4 Embarcadero Center, Suite 3400, San Francisco

STATE	CA	ZIP CODE	94111	COUNTRY	US

ENCLOSED APPLICATION PARTS (*check all that apply*)

X	Specification	*Number of Pages*	48	[ ]	Small Entity Statement
X	Drawings	*Number of Sheets*	3	[ ]	Other (*specify*):

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (*check one*)

			PROVISIONAL FILING FEE AMOUNT ($)	$150
X	A check or money order is enclosed to cover the Provisional filing fees			
X	The Commissioner is hereby authorized to charge any underpayment or overpayment to Deposit Account Number:	06-1300 (Order No. P-66818/WHD/MTK)		

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No.

[ ] Yes, the name of the U.S. Government Agency and the Government contract number are: ____________

Respectfully submitted,

SIGNATURE: [signature]

Date: August 18, 1998

TYPED or PRINTED NAME: Mark T. Kresnak, Ph.D.

REGISTRATION NO. (*if appropriate*): P-42,767

[ ] Additional inventors are being named on separately numbered sheets attached hereto.

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NOVEL POLYPEPTIDES HAVING SEQUENCE SIMILARITY TO FACTOR C AND NUCLEIC ACIDS ENCODING THE SAME

FIELD OF THE INVENTION

The present invention relates generally to the identification and isolation of novel DNA having homology to nucleic acid encoding factor C protein, and to the recombinant production of novel polypeptides, designated herein as "PRO1344" polypeptides.

BACKGROUND OF THE INVENTION

Extracellular proteins play an important role in the formation, differentiation and maintenance of multicellular organisms. The fate of many individual cells, e.g., proliferation, migration, differentiation, or interaction with other cells, is typically governed by information received from other cells and/or the immediate environment. This information is often transmitted by secreted polypeptides (for instance, mitogenic factors, survival factors, cytotoxic factors, differentiation factors, neuropeptides, and hormones) which are, in turn, received and interpreted by diverse cell receptors or membrane-bound proteins. These secreted polypeptides or signaling molecules normally pass through the cellular secretory pathway to reach their site of action in the extracellular environment.

Secreted proteins have various industrial applications, including pharmaceuticals, diagnostics, biosensors and bioreactors. Most protein drugs available at present, such as thrombolytic agents, interferons, interleukins, erythropoietins, colony stimulating factors, and various other cytokines, are secretory proteins. Their receptors, which are membrane proteins, also have potential as therapeutic or diagnostic agents. Efforts are being undertaken by both industry and academia to identify new, native secreted proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel secreted proteins. Examples of screening methods and techniques are described in the literature [see, for example, Klein et al., Proc. Natl. Acad. Sci., 93:7108-7113 (1996); U.S. Patent No. 5,536,637)].

Factor C is a protein that is intimately involved with the coagulation cascade in a variety of organisms. The coagulation cascade has been shown to involve numerous different intermediate proteins, including factor C, all of whose activity is essential to the proper functioning of this cascade. Abnormal coagulation cascade function can result in a variety of serious abnormalities and, as such, the activities of the coagulation cascade proteins is of particular interest. As such, efforts are currently being undertaken to identify novel polypeptides having homology to one or more of the coagulation cascade proteins.

We herein describe the identification and characterization of novel polypeptides having homology to factor C protein, designated herein as PRO1344 polypeptides.

SUMMARY OF THE INVENTION

A cDNA clone (DNA58723-1588) has been identified, having homology to nucleic acid encoding factor C that encodes a novel polypeptide, designated in the present application as "PRO1344".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO1344 polypeptide.

In one aspect, the isolated nucleic acid comprises DNA having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to (a) a DNA molecule encoding a PRO1344 polypeptide having the sequence of amino acid residues from about 1 or about 24 to about 720, inclusive of Figure 2 (SEQ ID NO:2), or (b) the complement of the DNA molecule of (a).

In another aspect, the invention concerns an isolated nucleic acid molecule encoding a PRO1344 polypeptide comprising DNA hybridizing to the complement of the nucleic acid between about nucleotides 26 or about 95 and about 2185, inclusive, of Figure 1 (SEQ ID NO:1). Preferably, hybridization occurs under stringent hybridization and wash conditions.

In a further aspect, the invention concerns an isolated nucleic acid molecule comprising DNA having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to (a) a DNA molecule encoding the same mature polypeptide encoded by the human protein cDNA in ATCC Deposit No. _____ (DNA58723-1588) or (b) the complement of the nucleic acid molecule of (a). In a preferred embodiment, the

nucleic acid comprises a DNA encoding the same mature polypeptide encoded by the human protein cDNA in ATCC Deposit No. _____ (DNA58723-1588).

In still a further aspect, the invention concerns an isolated nucleic acid molecule comprising (a) DNA encoding a polypeptide having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to the sequence of amino acid residues 1 or about 24 to about 720, inclusive of Figure 2 (SEQ ID NO:2), or (b) the complement of the DNA of (a).

In a further aspect, the invention concerns an isolated nucleic acid molecule having at least 10 nucleotides and produced by hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a PRO1344 polypeptide having the sequence of amino acid residues from 1 or about 24 to about 720, inclusive of Figure 2 (SEQ ID NO:2), or (b) the complement of the DNA molecule of (a), and, if the DNA molecule has at least about an 80 % sequence identity, prefereably at least about an 85% sequence identity, more preferably at least about a 90% sequence identity, most preferably at least about a 95% sequence identity to (a) or (b), isolating the test DNA molecule.

In a specific aspect, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO1344 polypeptide, with or without the N-terminal signal sequence and/or the initiating methionine, or is complementary to such encoding nucleic acid molecule. The signal peptide has been tentatively identified as extending from about amino acid position 1 to about amino acid position 23 in the sequence of Figure 2 (SEQ ID NO:2).

In another aspect, the invention concerns an isolated nucleic acid molecule comprising (a) DNA encoding a polypeptide scoring at least about 80% positives, preferably at least about 85% positives, more preferably at least about 90% positives, most preferably at least about 95% positives when compared with the amino acid sequence of residues 1 or about 24 to about 720, inclusive of Figure 2 (SEQ ID NO:2), or (b) the complement of the DNA of (a).

Another embodiment is directed to fragments of a PRO1344 polypeptide coding sequence that may find use as hybridization probes. Such nucleic acid fragments are from about 20 to about 80 nucleotides in length, preferably from about 20 to about 60 nucleotides in length, more preferably from about 20 to about 50 nucleotides in length and most preferably from about 20 to about 40 nucleotides in length and may be derived from the nucleotide sequence shown in Figure 1 (SEQ ID NO:1).

In another embodiment, the invention provides a vector comprising DNA encoding PRO1344 or its variants. The vector may comprise any of the isolated nucleic acid molecules hereinabove identified.

A host cell comprising such a vector is also provided. By way of example, the host cells may be CHO cells, *E. coli*, or yeast. A process for producing PRO1344 polypeptides is further provided and comprises culturing host cells under conditions suitable for expression of PRO1344 and recovering PRO1344 from the cell culture.

In another embodiment, the invention provides isolated PRO1344 polypeptide encoded by any of the isolated nucleic acid sequences hereinabove identified.

In a specific aspect, the invention provides isolated native sequence PRO1344 polypeptide, which in certain embodiments, includes an amino acid sequence comprising residues 1 or about 24 to about 720 of Figure 2 (SEQ ID NO:2).

In another aspect, the invention concerns an isolated PRO1344 polypeptide, comprising an amino acid sequence having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to the sequence of amino acid residues 1 or about 24 to about 720, inclusive of Figure 2 (SEQ ID NO:2).

In a further aspect, the invention concerns an isolated PRO1344 polypeptide, comprising an amino acid sequence scoring at least about 80% positives, preferably at least about 85% positives, more preferably at least about 90% positives, most preferably at least about 95% positives when compared with the amino acid sequence of residues 1 or about 24 to about 720, inclusive of Figure 2 (SEQ ID NO:2).

In yet another aspect, the invention concerns an isolated PRO1344 polypeptide, comprising the sequence of amino acid residues 1 or about 24 to about 720, inclusive of Figure 2 (SEQ ID NO:2), or a fragment thereof sufficient to provide a binding site for an anti-PRO1344 antibody. Preferably, the PRO1344 fragment retains a qualitative biological activity of a native PRO1344 polypeptide.

In a still further aspect, the invention provides a polypeptide produced by (i) hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a PRO1344 polypeptide having the sequence of amino acid residues from about 1 or about 24 to about 720, inclusive of Figure 2 (SEQ ID NO: 3), or (b) the complement of the DNA molecule of (a), and if the test DNA molecule has at least about an 80% sequence identity,

preferably at least about an 85% sequence identity, more preferably at least about a 90% sequence identity, most preferably at least about a 95% sequence identity to (a) or (b), (ii) culturing a host cell comprising the test DNA molecule under conditions suitable for expression of the polypeptide, and (iii) recovering the polypeptide from the cell culture.

In another embodiment, the invention provides chimeric molecules comprising a PRO1344 polypeptide fused to a heterologous polypeptide or amino acid sequence. An example of such a chimeric molecule comprises a PRO1344 polypeptide fused to an epitope tag sequence or a Fc region of an immunoglobulin.

In another embodiment, the invention provides an antibody which specifically binds to a PRO1344 polypeptide. Optionally, the antibody is a monoclonal antibody.

In yet another embodiment, the invention concerns agonists and antagonists of a native PRO1344 polypeptide. In a particular embodiment, the agonist or antagonist is an anti-PRO1344 antibody.

In a further embodiment, the invention concerns a method of identifying agonists or antagonists of a native PRO1344 polypeptide by contacting the native PRO1344 polypeptide with a candidate molecule and monitoring a biological activity mediated by said polypeptide.

In a still further embodiment, the invention concerns a composition comprising a PRO1344 polypeptide, or an agonist or antagonist as hereinabove defined, in combination with a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleotide sequence (SEQ ID NO:1) of a cDNA containing a nucleotide sequence (nucleotides 26-2185) encoding native sequence PRO1344, wherein the nucleotide sequence (SEQ ID NO:1) is a clone designated herein as "UNQ699" and/or "DNA58723-1588". Also presented in bold font and underlined are the positions of the start and stop codons at nucleotides 26 to 28 and 2186 to 2188, respectively.

Figure 2 shows the derived amino acid sequence (SEQ ID NO:2) of a native sequence PRO1344 polypeptide. Also shown are the approximate locations of various other important protein domains.

Figure 3 shows a nucleotide sequence designated herein as DNA33790 (SEQ ID NO:3).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. Definitions

The terms "PRO1344 polypeptide", "PRO1344 protein" and "PRO1344" when used herein encompass native sequence PRO1344 and PRO1344 variants (which are further defined herein). The PRO1344 may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant and/or synthetic methods.

A "native sequence PRO1344" comprises a polypeptide having the same amino acid sequence as a PRO1344 derived from nature. Such native sequence PRO1344 can be isolated from nature or can be produced by recombinant and/or synthetic means. The term "native sequence PRO1344" specifically encompasses naturally-occurring truncated or secreted forms (*e.g.*, an extracellular domain sequence), naturally-occurring variant forms (*e.g.*, alternatively spliced forms) and naturally-occurring allelic variants of the PRO1344. In one embodiment of the invention, the native sequence PRO1344 is a mature or full-length native sequence PRO1344 comprising amino acids 1 to 720 of Figure 2 (SEQ ID NO:2).

"PRO1344 variant" means an active PRO1344 as defined below having at least about 80% amino acid sequence identity with the amino acid sequence of residues 1 or about 24 to 720 of the PRO1344 polypeptide having the deduced amino acid sequence shown in Figure 2 (SEQ ID NO:2). Such PRO1344 variants include, for instance, PRO1344 polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus, as well as within one or more internal domains, of the sequence of Figure 2 (SEQ ID NO:2). Ordinarily, a PRO1344 variant will have at least about 80% amino acid sequence identity, more preferably at least about 85% amino acid sequence identity, even more preferably at least about 90% amino acid sequence identity, and most preferably at least about 95% sequence identity with the amino acid sequence of residues 1 or about 24 to 720 of Figure 2 (SEQ ID NO:2). Variants do not encompass the native sequence.

"Percent (%) amino acid sequence identity" with respect to the PRO1344 sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the PRO1344 sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. The % identity values used herein are generated by WU-BLAST-2 which was obtained from [Altschul et al., Methods in Enzymology, 266: 460-480 (1996);

http://blast.wustl/edu/blast/README.html]. WU-BLAST-2 uses several search parameters, most of which are set to the default values. The adjustable parameters are set with the following values: overlap span =1, overlap fraction = 0.125, word threshold (T) = 11. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched; however, the values may be adjusted to increase sensitivity. A % amino acid sequence identity value is determined by the number of matching identical residues divided by the total number of residues of the "longer" sequence in the aligned region. The "longer" sequence is the one having the most actual residues in the aligned region (gaps introduced by WU-Blast-2 to maximize the alignment score are ignored).

The term "positives", in the context of sequence comparison performed as described above, includes residues in the sequences compared that are not identical but have similar properties (e.g. as a result of conservative substitutions). The % value of positives is determined by the fraction of residues scoring a positive value in the BLOSUM 62 matrix divided by the total number of residues in the longer sequence, as defined above.

In a similar manner, "percent (%) nucleic acid sequence identity" with respect to the coding sequence of the PRO1344 polypeptides identified herein is defined as the percentage of nucleotide residues in a candidate sequence that are identical with the nucleotide residues in the PRO1344 coding sequence. The identity values used herein were generated by the BLASTN module of WU-BLAST-2 set to the default parameters, with overlap span and overlap fraction set to 1 and 0.125, respectively.

"Isolated," when used to describe the various polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the PRO1344 natural

environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

An "isolated" nucleic acid molecule encoding a PRO1344 polypeptide is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the PRO1344-encoding nucleic acid. An isolated PRO1344-encoding nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the PRO1344-encoding nucleic acid molecule as it exists in natural cells. However, an isolated nucleic acid molecule encoding a PRO1344 polypeptide includes PRO1344-encoding nucleic acid molecules contained in cells that ordinarily express PRO1344 where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

The term "antibody" is used in the broadest sense and specifically covers single anti-PRO1344 monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies) and anti-PRO1344 antibody compositions with polyepitopic specificity. The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical

except for possible naturally-occurring mutations that may be present in minor amounts.

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel *et al.*, Current Protocols in Molecular Biology, Wiley Interscience Publishers, (1995).

"Stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 μg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

"Moderately stringent conditions" may be identified as described by Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and %SDS) less stringent that those described above. An example of moderately stringent conditions is overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/mL denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan

will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising a PRO1344 polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (i.e., is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

"Active" or "activity" for the purposes herein refers to form(s) of PRO1344 which retain the biologic and/or immunologic activities of native or naturally-occurring PRO1344.

The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native PRO1344 polypeptide disclosed herein. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics a biological activity of a native PRO1344 polypeptide disclosed herein. Suitable agonist or antagonist molecules specifically include agonist or antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native PRO1344 polypeptides, peptides, small organic molecules, etc.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as

well as those prone to have the disorder or those in whom the disorder is to be prevented.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cows, horses, sheep, pigs, etc. Preferably, the mammal is human.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

II. Compositions and Methods of the Invention

A. Full-length PRO1344 Polypeptide

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO1344 (UNQ699). In particular, cDNA encoding a PRO1344 polypeptide has been identified and isolated, as disclosed in further detail in the Examples below. It is noted that proteins produced in separate expression rounds may be given different PRO numbers but the UNQ number is unique for any given DNA and the encoded protein, and will not be changed. However, for sake of simplicity, in the present specification the protein encoded by DNA58723-1588 as well as all further native homologues and variants included in the foregoing definition of PRO1344, will be referred to as "PRO1344", regardless of their origin or mode of preparation.

Using the WU-BLAST2 sequence alignment computer program, it has been found that a full-length native sequence PRO1344 (shown in Figure 2 and SEQ ID NO:2) has about 34% amino acid sequence identity with the factor C protein of Carcinoscorpius rotundicauda. Accordingly, it is presently believed that PRO1344 disclosed in the present application is a newly identified factor C protein and may possess activity typical of that protein.

B. PRO1344 Variants

In addition to the full-length native sequence PRO1344 polypeptides described herein, it is contemplated that PRO1344 variants can be prepared. PRO1344 variants can be prepared by introducing appropriate nucleotide changes into the PRO1344 DNA, and/or by synthesis

of the desired PRO1344 polypeptide. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of the PRO1344, such as changing the number or position of glycosylation sites or altering the membrane anchoring characteristics.

Variations in the native full-length sequence PRO1344 or in various domains of the PRO1344 described herein, can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Patent No. 5,364,934. Variations may be a substitution, deletion or insertion of one or more codons encoding the PRO1344 that results in a change in the amino acid sequence of the PRO1344 as compared with the native sequence PRO1344. Optionally the variation is by substitution of at least one amino acid with any other amino acid in one or more of the domains of the PRO1344. Guidance in determining which amino acid residue may be inserted, substituted or deleted without adversely affecting the desired activity may be found by comparing the sequence of the PRO1344 with that of homologous known protein molecules and minimizing the number of amino acid sequence changes made in regions of high homology. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, i.e., conservative amino acid replacements. Insertions or deletions may optionally be in the range of 1 to 5 amino acids. The variation allowed may be determined by systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity in the *in vitro* assay described in the Examples below.

The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter et al., Nucl. Acids Res., 13:4331 (1986); Zoller et al., Nucl. Acids Res., 10:6487 (1987)], cassette mutagenesis [Wells et al., Gene, 34:315 (1985)], restriction selection mutagenesis [Wells et al., Philos. Trans. R. Soc. London SerA, 317:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the PRO1344 variant DNA.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-

chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant [Cunningham and Wells, Science, 244: 1081-1085 (1989)]. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions [Creighton, The Proteins, (W.H. Freeman & Co., N.Y.); Chothia, J. Mol. Biol., 150:1 (1976)]. If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

C. Modifications of PRO1344

Covalent modifications of PRO1344 are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of a PRO1344 polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C- terminal residues of the PRO1344. Derivatization with bifunctional agents is useful, for instance, for crosslinking PRO1344 to a water-insoluble support matrix or surface for use in the method for purifying anti-PRO1344 antibodies, and vice-versa. Commonly used crosslinking agents include, *e.g.*, 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate.

Other modifications include deamidation of glutaminyl and asparaginyl residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α-amino groups of lysine, arginine, and histidine side chains [T.E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)], acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the PRO1344 polypeptide included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in native sequence PRO1344 (either by removing the underlying glycosylation site or by deleting the glycosylation by chemical and/or enzymatic means), and/or adding one or more glycosylation sites that are not present in the native sequence PRO1344. In addition, the phrase includes qualitative changes in the glycosylation

of the native proteins, involving a change in the nature and proportions of the various carbohydrate moieties present.

Addition of glycosylation sites to the PRO1344 polypeptide may be accomplished by altering the amino acid sequence. The alteration may be made, for example, by the addition of, or substitution by, one or more serine or threonine residues to the native sequence PRO1344 (for O-linked glycosylation sites). The PRO1344 amino acid sequence may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the PRO1344 polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

Another means of increasing the number of carbohydrate moieties on the PRO1344 polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Such methods are described in the art, e.g., in WO 87/05330 published 11 September 1987, and in Aplin and Wriston, CRC Crit. Rev. Biochem., pp. 259-306 (1981).

Removal of carbohydrate moieties present on the PRO1344 polypeptide may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. Chemical deglycosylation techniques are known in the art and described, for instance, by Hakimuddin, et al., Arch. Biochem. Biophys., 259:52 (1987) and by Edge et al., Anal. Biochem., 118:131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al., Meth. Enzymol., 138:350 (1987).

Another type of covalent modification of PRO1344 comprises linking the PRO1344 polypeptide to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol (PEG), polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

The PRO1344 of the present invention may also be modified in a way to form a chimeric molecule comprising PRO1344 fused to another, heterologous polypeptide or amino acid sequence.

In one embodiment, such a chimeric molecule comprises a fusion of the PRO1344 with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl- terminus of the PRO1344. The presence of such epitope-tagged forms of the PRO1344 can be detected using an antibody

against the tag polypeptide. Also, provision of the epitope tag enables the PRO1344 to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field et al., Mol. Cell. Biol., 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al., Molecular and Cellular Biology, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky et al., Protein Engineering, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag-peptide [Hopp et al., BioTechnology, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin et al., Science, 255:192-194 (1992)]; an α-tubulin epitope peptide [Skinner et al., J. Biol. Chem., 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth et al., Proc. Natl. Acad. Sci. USA, 87:6393-6397 (1990)].

In an alternative embodiment, the chimeric molecule may comprise a fusion of the PRO1344 with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule (also referred to as an "immunoadhesin"), such a fusion could be to the Fc region of an IgG molecule. The Ig fusions preferably include the substitution of a soluble (transmembrane domain deleted or inactivated) form of a PRO1344 polypeptide in place of at least one variable region within an Ig molecule. In a particularly preferred embodiment, the immunoglobulin fusion includes the hinge, CH2 and CH3, or the hinge, CH1, CH2 and CH3 regions of an IgG1 molecule. For the production of immunoglobulin fusions see also US Patent No. 5,428,130 issued June 27, 1995.

D. Preparation of PRO1344

The description below relates primarily to production of PRO1344 by culturing cells transformed or transfected with a vector containing PRO1344 nucleic acid. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare PRO1344. For instance, the PRO1344 sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques [see, e.g., Stewart et al., Solid-Phase Peptide Synthesis, W.H. Freeman Co., San Francisco, CA (1969); Merrifield, J. Am. Chem. Soc., 85:2149-2154 (1963)]. *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance,

using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of the PRO1344 may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the full-length PRO1344.

1. Isolation of DNA Encoding PRO1344

DNA encoding PRO1344 may be obtained from a cDNA library prepared from tissue believed to possess the PRO1344 mRNA and to express it at a detectable level. Accordingly, human PRO1344 DNA can be conveniently obtained from a cDNA library prepared from human tissue, such as described in the Examples. The PRO1344-encoding gene may also be obtained from a genomic library or by oligonucleotide synthesis.

Libraries can be screened with probes (such as antibodies to the PRO1344 or oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described in Sambrook et al., Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the gene encoding PRO1344 is to use PCR methodology [Sambrook et al., supra; Dieffenbach et al., PCR Primer: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1995)].

The Examples below describe techniques for screening a cDNA library. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The oligonucleotide is preferably labeled such that it can be detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like ^{32}P-labeled ATP, biotinylation or enzyme labeling. Hybridization conditions, including moderate stringency and high stringency, are provided in Sambrook et al., supra.

Sequences identified in such library screening methods can be compared and aligned to other known sequences deposited and available in public databases such as GenBank or other private sequence databases. Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined through sequence alignment using computer software programs such as ALIGN, DNAstar, BLAST, BLAST2 and INHERIT which employ various algorithms to measure homology.

Nucleic acid having protein coding sequence may be obtained by screening selected

cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in Sambrook et al., supra, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

2. Selection and Transformation of Host Cells

Host cells are transfected or transformed with expression or cloning vectors described herein for PRO1344 production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in Mammalian Cell Biotechnology: a Practical Approach, M. Butler, ed. (IRL Press, 1991) and Sambrook et al., supra.

Methods of transfection are known to the ordinarily skilled artisan, for example, $CaPO_4$ and electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook et al., supra, or electroporation is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw et al., Gene, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) can be employed. General aspects of mammalian cell host system transformations have been described in U.S. Patent No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen et al., J. Bact., 130:946 (1977) and Hsiao et al., Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, *e.g.*, polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown et al., Methods in Enzymology, 185:527-537 (1990) and Mansour et al., Nature, 336:348-352 (1988).

Suitable host cells for cloning or expressing the DNA in the vectors herein include

prokaryote, yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *E. coli*. Various *E. coli* strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635).

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for PRO1344-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism.

Suitable host cells for the expression of glycosylated PRO1344 are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as Drosophila S2 and Spodoptera Sf9, as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol., 36:59 (1977)); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77:4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243-251 (1980)); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor (MMT 060562, ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

3. Selection and Use of a Replicable Vector

The nucleic acid (*e.g.*, cDNA or genomic DNA) encoding PRO1344 may be inserted into a replicable vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally include, but are not limited to, one or more of a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

The PRO1344 may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the PRO1344-encoding DNA that is inserted into the vector. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, *e.g.*, the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders, the latter described in U.S. Patent No. 5,010,182), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells.

Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*, the gene encoding D-alanine racemase for *Bacilli*.

An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the PRO1344-encoding nucleic acid, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described by Urlaub et al., Proc. Natl. Acad. Sci. USA, *77*:4216 (1980). A suitable selection gene for use in yeast is the *trp*1 gene present in the yeast plasmid YRp7 [Stinchcomb et al., Nature, 282:39 (1979); Kingsman et al., Gene, 7:141 (1979); Tschemper et al., Gene, 10:157 (1980)]. The *trp*1 gene

provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 [Jones, Genetics, 85:12 (1977)].

Expression and cloning vectors usually contain a promoter operably linked to the PRO1344-encoding nucleic acid sequence to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known. Promoters suitable for use with prokaryotic hosts include the β-lactamase and lactose promoter systems [Chang et al., Nature, 275:615 (1978); Goeddel et al., Nature, 281:544 (1979)], alkaline phosphatase, a tryptophan (trp) promoter system [Goeddel, Nucleic Acids Res., 8:4057 (1980); EP 36,776], and hybrid promoters such as the tac promoter [deBoer et al., Proc. Natl. Acad. Sci. USA, 80:21-25 (1983)]. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding PRO1344.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman et al., J. Biol. Chem., 255:2073 (1980)] or other glycolytic enzymes [Hess et al., J. Adv. Enzyme Reg., 7:149 (1968); Holland, Biochemistry, 17:4900 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657.

PRO1344 transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible with the host cell systems.

Transcription of a DNA encoding the PRO1344 by higher eukaryotes may be increased

by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the PRO1344 coding sequence, but is preferably located at a site 5' from the promoter.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding PRO1344.

Still other methods, vectors, and host cells suitable for adaptation to the synthesis of PRO1344 in recombinant vertebrate cell culture are described in Gething et al., Nature, 293:620-625 (1981); Mantei et al., Nature, 281:40-46 (1979); EP 117,060; and EP 117,058.

4. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA [Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 (1980)], dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for

immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence PRO1344 polypeptide or against a synthetic peptide based on the DNA sequences provided herein or against exogenous sequence fused to PRO1344 DNA and encoding a specific antibody epitope.

5. Purification of Polypeptide

Forms of PRO1344 may be recovered from culture medium or from host cell lysates. If membrane-bound, it can be released from the membrane using a suitable detergent solution (*e.g.* Triton-X 100) or by enzymatic cleavage. Cells employed in expression of PRO1344 can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

It may be desired to purify PRO1344 from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the PRO1344. Various methods of protein purification may be employed and such methods are known in the art and described for example in Deutscher, Methods in Enzymology, 182 (1990); Scopes, Protein Purification: Principles and Practice, Springer-Verlag, New York (1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular PRO1344 produced.

E. Uses for PRO1344

Nucleotide sequences (or their complement) encoding PRO1344 have various applications in the art of molecular biology, including uses as hybridization probes, in chromosome and gene mapping and in the generation of anti-sense RNA and DNA. PRO1344 nucleic acid will also be useful for the preparation of PRO1344 polypeptides by the recombinant techniques described herein.

The full-length native sequence PRO1344 gene (SEQ ID NO:1), or portions thereof, may be used as hybridization probes for a cDNA library to isolate the full-length PRO1344

gene or to isolate still other genes (for instance, those encoding naturally-occurring variants of PRO1344 or PRO1344 from other species) which have a desired sequence identity to the PRO1344 sequence disclosed in Fig. 1 (SEQ ID NO:1). Optionally, the length of the probes will be about 20 to about 50 bases. The hybridization probes may be derived from the nucleotide sequence of SEQ ID NO:1 or from genomic sequences including promoters, enhancer elements and introns of native sequence PRO1344. By way of example, a screening method will comprise isolating the coding region of the PRO1344 gene using the known DNA sequence to synthesize a selected probe of about 40 bases. Hybridization probes may be labeled by a variety of labels, including radionucleotides such as ^{32}P or ^{35}S, or enzymatic labels such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems. Labeled probes having a sequence complementary to that of the PRO1344 gene of the present invention can be used to screen libraries of human cDNA, genomic DNA or mRNA to determine which members of such libraries the probe hybridizes to. Hybridization techniques are described in further detail in the Examples below.

The probes may also be employed in PCR techniques to generate a pool of sequences for identification of closely related PRO1344 coding sequences.

Nucleotide sequences encoding a PRO1344 can also be used to construct hybridization probes for mapping the gene which encodes that PRO1344 and for the genetic analysis of individuals with genetic disorders. The nucleotide sequences provided herein may be mapped to a chromosome and specific regions of a chromosome using known techniques, such as *in situ* hybridization, linkage analysis against known chromosomal markers, and hybridization screening with libraries.

When the coding sequences for PRO1344 encode a protein which binds to another protein (example, where the PRO1344 is a receptor), the PRO1344 can be used in assays to identify the other proteins or molecules involved in the binding interaction. By such methods, inhibitors of the receptor/ligand binding interaction can be identified. Proteins involved in such binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction. Also, the receptor PRO1344 can be used to isolate correlative ligand(s). Screening assays can be designed to find lead compounds that mimic the biological activity of a native PRO1344 or a receptor for PRO1344. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules

contemplated include synthetic organic or inorganic compounds. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays and cell based assays, which are well characterized in the art.

Nucleic acids which encode PRO1344 or its modified forms can also be used to generate either transgenic animals or "knock out" animals which, in turn, are useful in the development and screening of therapeutically useful reagents. A transgenic animal (e.g., a mouse or rat) is an animal having cells that contain a transgene, which transgene was introduced into the animal or an ancestor of the animal at a prenatal, e.g., an embryonic stage. A transgene is a DNA which is integrated into the genome of a cell from which a transgenic animal develops. In one embodiment, cDNA encoding PRO1344 can be used to clone genomic DNA encoding PRO1344 in accordance with established techniques and the genomic sequences used to generate transgenic animals that contain cells which express DNA encoding PRO1344. Methods for generating transgenic animals, particularly animals such as mice or rats, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009. Typically, particular cells would be targeted for PRO1344 transgene incorporation with tissue-specific enhancers. Transgenic animals that include a copy of a transgene encoding PRO1344 introduced into the germ line of the animal at an embryonic stage can be used to examine the effect of increased expression of DNA encoding PRO1344. Such animals can be used as tester animals for reagents thought to confer protection from, for example, pathological conditions associated with its overexpression. In accordance with this facet of the invention, an animal is treated with the reagent and a reduced incidence of the pathological condition, compared to untreated animals bearing the transgene, would indicate a potential therapeutic intervention for the pathological condition.

Alternatively, non-human homologues of PRO1344 can be used to construct a PRO1344 "knock out" animal which has a defective or altered gene encoding PRO1344 as a result of homologous recombination between the endogenous gene encoding PRO1344 and altered genomic DNA encoding PRO1344 introduced into an embryonic cell of the animal. For example, cDNA encoding PRO1344 can be used to clone genomic DNA encoding PRO1344 in accordance with established techniques. A portion of the genomic DNA encoding PRO1344 can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector [see e.g., Thomas and

Capecchi, Cell, 51:503 (1987) for a description of homologous recombination vectors]. The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected [see e.g., Li et al., Cell, 69:915 (1992)]. The selected cells are then injected into a blastocyst of an animal (e.g., a mouse or rat) to form aggregation chimeras [see e.g., Bradley, in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock out" animal. Progeny harboring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized for instance, for their ability to defend against certain pathological conditions and for their development of pathological conditions due to absence of the PRO1344 polypeptide.

Nucleic acid encoding the PRO1344 polypeptides may also be used in gene therapy. In gene therapy applications, genes are introduced into cells in order to achieve *in vivo* synthesis of a therapeutically effective genetic product, for example for replacement of a defective gene. "Gene therapy" includes both conventional gene therapy where a lasting effect is achieved by a single treatment, and the administration of gene therapeutic agents, which involves the one time or repeated administration of a therapeutically effective DNA or mRNA. Antisense RNAs and DNAs can be used as therapeutic agents for blocking the expression of certain genes *in vivo*. It has already been shown that short antisense oligonucleotides can be imported into cells where they act as inhibitors, despite their low intracellular concentrations caused by their restricted uptake by the cell membrane. (Zamecnik *et al.*, Proc. Natl. Acad. Sci. USA 83, 4143-4146 [1986]). The oligonucleotides can be modified to enhance their uptake, e.g. by substituting their negatively charged phosphodiester groups by uncharged groups.

There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro*, or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. The currently preferred *in vivo* gene transfer techniques include transfection with viral

(typically retroviral) vectors and viral coat protein-liposome mediated transfection (Dzau *et al.*, Trends in Biotechnology 11, 205-210 [1993]). In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cell, etc. Where liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, e.g. capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu *et al.*, J. Biol. Chem. 262, 4429-4432 (1987); and Wagner *et al.*, Proc. Natl. Acad. Sci. USA 87, 3410-3414 (1990). For review of gene marking and gene therapy protocols see Anderson *et al.*, Science 256, 808-813 (1992).

The PRO1344 polypeptides of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the PRO1344 product hereof is combined in admixture with a pharmaceutically acceptable carrier vehicle. Therapeutic formulations are prepared for storage by mixing the active ingredient having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone, amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, Pluronics or PEG.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution.

Therapeutic compositions herein generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by

a hypodermic injection needle.

The route of administration is in accord with known methods, e.g. injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial or intralesional routes, topical administration, or by sustained release systems. --

Dosages and desired drug concentrations of pharmaceutical compositions of the present invention may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary physician. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W. "The use of interspecies scaling in toxicokinetics" In Toxicokinetics and New Drug Development, Yacobi *et al.*, Eds., Pergamon Press, New York 1989, pp. 42-96.

F. Anti-PRO1344 Antibodies

The present invention further provides anti-PRO1344 antibodies. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies.

1. Polyclonal Antibodies

The anti-PRO1344 antibodies may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the PRO1344 polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

2. Monoclonal Antibodies

The anti-PRO1344 antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

The immunizing agent will typically include the PRO1344 polypeptide or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103]. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Rockville, Maryland. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63].

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against PRO1344. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by

immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980). --

After the desired hybridoma cells are identified, the clones may be subcloned by limiting-dilution procedures and grown by standard methods [Goding, supra]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; Morrison et al., supra] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is

truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art.

3. Human and Humanized Antibodies

The anti-PRO1344 antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., Nature,

321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)]. The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985) and Boerner et al., J. Immunol., 147(1):86-95 (1991)]. Similarly, human antibodies can be made by introducing of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks *et al.*, Bio/Technology 10, 779-783 (1992); Lonberg *et al.*, Nature 368 856-859 (1994); Morrison, Nature 368, 812-13 (1994); Fishwild *et al.*, Nature Biotechnology 14, 845-51 (1996); Neuberger, Nature Biotechnology 14, 826 (1996); Lonberg and Huszar, Intern. Rev. Immunol. 13 65-93 (1995).

4. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the PRO1344, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different

specificities [Milstein and Cuello, Nature, 305:537-539 (1983)]. Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker et al., EMBO J., 10:3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 121:210 (1986).

5. Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells [U.S. Patent No. 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/200373; EP 03089]. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

G. Uses for anti-PRO1344 Antibodies

The anti-PRO1344 antibodies of the invention have various utilities. For example, anti-PRO1344 antibodies may be used in diagnostic assays for PRO1344, *e.g.*, detecting its expression in specific cells, tissues, or serum. Various diagnostic assay techniques known in

the art may be used, such as competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays conducted in either heterogeneous or homogeneous phases [Zola, Monoclonal Antibodies: A Manual of Techniques, CRC Press, Inc. (1987) pp. 147-158]. The antibodies used in the diagnostic assays can be labeled with a detectable moiety. The detectable moiety should be capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase. Any method known in the art for conjugating the antibody to the detectable moiety may be employed, including those methods described by Hunter et al., Nature, 144:945 (1962); David et al., Biochemistry, 13:1014 (1974); Pain et al., J. Immunol. Meth., 40:219 (1981); and Nygren, J. Histochem. and Cytochem., 30:407 (1982).

Anti-PRO1344 antibodies also are useful for the affinity purification of PRO1344 from recombinant cell culture or natural sources. In this process, the antibodies against PRO1344 are immobilized on a suitable support, such a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody then is contacted with a sample containing the PRO1344 to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the PRO1344, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent that will release the PRO1344 from the antibody.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

EXAMPLES

Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection, Manassas, VA.

EXAMPLE 1

Isolation of cDNA clones Encoding Human PRO1344

The extracellular domain (ECD) sequences (including the secretion signal sequence, if any) from about 950 known secreted proteins from the Swiss-Prot public database were used to search EST databases. The EST databases included public EST databases (e.g., GenBank), and a proprietary EST database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 [Altschul et al., Methods in Enzymology, 266:460-480 (1996)] as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequences. Those comparisons resulting in a BLAST score of 70 (or in some cases, 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington).

A consensus DNA sequence was assembled relative to other EST sequences using phrap. This consensus sequence is shown in Figure 3 (SEQ ID NO:3) and is herein designated DNA33790. This sequence corresponds to Incyte EST clone no. 2210910. Based on the DNA33790 consensus sequence shown in Figure 3 (SEQ ID NO:3), oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO1344. Forward and reverse PCR primers generally range from 20 to 30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probe sequences are typically 40-55 bp in length. In some cases, additional oligonucleotides are synthesized when the consensus sequence is greater than about 1-1.5kbp. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel et al., Current Protocols in Molecular Biology, *supra*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

PCR primers (forward and reverse) were synthesized:

forward PCR primer (33790.f1) 5'-AGGTTCGTGATGGAGACAACCGCG-3' (SEQ ID NO:4)

reverse PCR primer (33790.r1) 5'-TGTCAAGGACGCACTGCCGTCATG-3' (SEQ ID NO:5)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the

consensus DNA33790 sequence which had the following nucleotide sequence

hybridization probe (33790.p1)

5'-TGGCCAGATCATCAAGCGTGTCTGTGGCAACGAGCGGCCAGCTCCTATCC-3'

(SEQ ID NO:6) --

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO1344 gene using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal kidney tissue. The cDNA libraries used to isolated the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., Science, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO1344 (designated herein as DNA58723-1588 [Figure 1, SEQ ID NO: 1]; (UNQ699) and the derived protein sequence for PRO1344.

The entire nucleotide sequence of UNQ699 (DNA58723-1588) is shown in Figure 1 (SEQ ID NO:1). Clone UNQ699 (DNA58723-1588) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 26-28 and ending at the stop codon at nucleotide positions 2186-2188 (Figure 1). The predicted polypeptide precursor is 720 amino acids long (Figure 2). The full-length PRO1344 protein shown in Figure 2 has an estimated molecular weight of about 80,199 daltons and a pI of about 7.77. Analysis of the full-length PRO1344 sequence shown in Figure 2 (SEQ ID NO:2) evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 23, an EGF-like domain cysteine protein signature sequence from about amino acid 260 to about amino acid 271, potential N-glycosylation sites from about amino acid 96 to about amino acid 99, from about amino acid 279 to about amino acid 282, from about amino acid 316 to about amino acid 319, from about amino acid 451 to about amino acid 454 and from about amino acid 614 to about amino acid 617, an amino acid sequence block having homology to serine proteases,

trypsin family from about amino acid 489 to about amino acid 505 and a CUB domain protein profile sequence from about amino acid 150 to about amino acid 166. Clone UNQ699 (DNA58723-1588) has been deposited with ATCC on August 18, 1998 and is assigned ATCC deposit no. _____.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using a WU-BLAST2 sequence alignment analysis of the full-length sequence shown in Figure 2 (SEQ ID NO:2), evidenced significant homology between the PRO1344 amino acid sequence and the following Dayhoff sequences: S77063_1, CRAR_MOUSE, P_R74775, P_P90070, P_R09217, P_P70475, HSBMP16_1 and U50330_1.

EXAMPLE 2

Use of PRO1344 as a hybridization probe

The following method describes use of a nucleotide sequence encoding PRO1344 as a hybridization probe.

DNA comprising the coding sequence of full-length or mature PRO1344 (as shown in Figure 1, SEQ ID NO:1) is employed as a probe to screen for homologous DNAs (such as those encoding naturally-occurring variants of PRO1344) in human tissue cDNA libraries or human tissue genomic libraries.

Hybridization and washing of filters containing either library DNAs is performed under the following high stringency conditions. Hybridization of radiolabeled PRO1344-derived probe to the filters is performed in a solution of 50% formamide, 5x SSC, 0.1% SDS, 0.1% sodium pyrophosphate, 50 mM sodium phosphate, pH 6.8, 2x Denhardt's solution, and 10% dextran sulfate at 42°C for 20 hours. Washing of the filters is performed in an aqueous solution of 0.1x SSC and 0.1% SDS at 42°C.

DNAs having a desired sequence identity with the DNA encoding full-length native sequence PRO1344 can then be identified using standard techniques known in the art.

EXAMPLE 3

Expression of PRO1344 in *E. coli*

This example illustrates preparation of an unglycosylated form of PRO1344 by recombinant expression in *E. coli*.

The DNA sequence encoding PRO1344 (SEQ ID NO:1) is initially amplified using

selected PCR primers. The primers should contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector. A variety of expression vectors may be employed. An example of a suitable vector is pBR322 (derived from *E. coli*; see Bolivar et al., Gene, 2:95 (1977)) which contains genes for ampicillin and tetracycline resistance. The vector is digested with restriction enzyme and dephosphorylated. The PCR amplified sequences are then ligated into the vector. The vector will preferably include sequences which encode for an antibiotic resistance gene, a trp promoter, a polyhis leader (including the first six STII codons, polyhis sequence, and enterokinase cleavage site), the PRO1344 coding region, lambda transcriptional terminator, and an argU gene.

The ligation mixture is then used to transform a selected *E. coli* strain using the methods described in Sambrook et al., supra. Transformants are identified by their ability to grow on LB plates and antibiotic resistant colonies are then selected. Plasmid DNA can be isolated and confirmed by restriction analysis and DNA sequencing.

Selected clones can be grown overnight in liquid culture medium such as LB broth supplemented with antibiotics. The overnight culture may subsequently be used to inoculate a larger scale culture. The cells are then grown to a desired optical density, during which the expression promoter is turned on.

After culturing the cells for several more hours, the cells can be harvested by centrifugation. The cell pellet obtained by the centrifugation can be solubilized using various agents known in the art, and the solubilized PRO1344 protein can then be purified using a metal chelating column under conditions that allow tight binding of the protein.

EXAMPLE 4

Expression of PRO1344 in mammalian cells

This example illustrates preparation of a potentially glycosylated form of PRO1344 by recombinant expression in mammalian cells.

The vector, pRK5 (see EP 307,247, published March 15, 1989), is employed as the expression vector. Optionally, the PRO1344 DNA is ligated into pRK5 with selected restriction enzymes to allow insertion of the PRO1344 DNA using ligation methods such as described in Sambrook et al., supra. The resulting vector is called pRK5-PRO1344.

In one embodiment, the selected host cells may be 293 cells. Human 293 cells (ATCC CCL 1573) are grown to confluence in tissue culture plates in medium such as DMEM

supplemented with fetal calf serum and optionally, nutrient components and/or antibiotics. About 10 μg pRK5-PRO1344 DNA is mixed with about 1 μg DNA encoding the VA RNA gene [Thimmappaya et al., Cell, 31:543 (1982)] and dissolved in 500 μl of 1 mM Tris-HCl, 0.1 mM EDTA, 0.227 M $CaCl_2$. To this mixture is added, dropwise, 500 μl of 50 mM HEPES (pH 7.35), 280 mM NaCl, 1.5 mM $NaPO_4$, and a precipitate is allowed to form for 10 minutes at 25°C. The precipitate is suspended and added to the 293 cells and allowed to settle for about four hours at 37°C. The culture medium is aspirated off and 2 ml of 20% glycerol in PBS is added for 30 seconds. The 293 cells are then washed with serum free medium, fresh medium is added and the cells are incubated for about 5 days.

Approximately 24 hours after the transfections, the culture medium is removed and replaced with culture medium (alone) or culture medium containing 200 μCi/ml ^{35}S-cysteine and 200 μCi/ml ^{35}S-methionine. After a 12 hour incubation, the conditioned medium is collected, concentrated on a spin filter, and loaded onto a 15% SDS gel. The processed gel may be dried and exposed to film for a selected period of time to reveal the presence of PRO1344 polypeptide. The cultures containing transfected cells may undergo further incubation (in serum free medium) and the medium is tested in selected bioassays.

In an alternative technique, PRO1344 may be introduced into 293 cells transiently using the dextran sulfate method described by Somparyrac et al., Proc. Natl. Acad. Sci., 12:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700 μg pRK5-PRO1344 DNA is added. The cells are first concentrated from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20% glycerol for 90 seconds, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5 μg/ml bovine insulin and 0.1 μg/ml bovine transferrin. After about four days, the conditioned media is centrifuged and filtered to remove cells and debris. The sample containing expressed PRO1344 can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography.

In another embodiment, PRO1344 can be expressed in CHO cells. The pRK5-PRO1344 can be transfected into CHO cells using known reagents such as $CaPO_4$ or DEAE-dextran. As described above, the cell cultures can be incubated, and the medium replaced with culture medium (alone) or medium containing a radiolabel such as ^{35}S-methionine. After determining the presence of PRO1344 polypeptide, the culture medium may be replaced with

serum free medium. Preferably, the cultures are incubated for about 6 days, and then the conditioned medium is harvested. The medium containing the expressed PRO1344 can then be concentrated and purified by any selected method.

Epitope-tagged PRO1344 may also be expressed in host CHO cells. The PRO1344 may be subcloned out of the pRK5 vector. The subclone insert can undergo PCR to fuse in frame with a selected epitope tag such as a poly-his tag into a Baculovirus expression vector. The poly-his tagged PRO1344 insert can then be subcloned into a SV40 driven vector containing a selection marker such as DHFR for selection of stable clones. Finally, the CHO cells can be transfected (as described above) with the SV40 driven vector. Labeling may be performed, as described above, to verify expression. The culture medium containing the expressed poly-His tagged PRO1344 can then be concentrated and purified by any selected method, such as by Ni^{2+}-chelate affinity chromatography.

EXAMPLE 5

Expression of PRO1344 in Yeast

The following method describes recombinant expression of PRO1344 in yeast.

First, yeast expression vectors are constructed for intracellular production or secretion of PRO1344 from the ADH2/GAPDH promoter. DNA encoding PRO1344 and the promoter is inserted into suitable restriction enzyme sites in the selected plasmid to direct intracellular expression of PRO1344. For secretion, DNA encoding PRO1344 can be cloned into the selected plasmid, together with DNA encoding the ADH2/GAPDH promoter, a native PRO1344 signal peptide or other mammalian signal peptide, or, for example, a yeast alpha-factor or invertase secretory signal/leader sequence, and linker sequences (if needed) for expression of PRO1344.

Yeast cells, such as yeast strain AB110, can then be transformed with the expression plasmids described above and cultured in selected fermentation media. The transformed yeast supernatants can be analyzed by precipitation with 10% trichloroacetic acid and separation by SDS-PAGE, followed by staining of the gels with Coomassie Blue stain.

Recombinant PRO1344 can subsequently be isolated and purified by removing the yeast cells from the fermentation medium by centrifugation and then concentrating the medium using selected cartridge filters. The concentrate containing PRO1344 may further be purified using selected column chromatography resins.

EXAMPLE 6

Expression of PRO1344 in Baculovirus-Infected Insect Cells

The following method describes recombinant expression of PRO1344 in Baculovirus-infected insect cells.

The sequence coding for PRO1344 is fused upstream of an epitope tag contained within a baculovirus expression vector. Such epitope tags include poly-his tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pVL1393 (Novagen). Briefly, the sequence encoding PRO1344 or the desired portion of the coding sequence of PRO1344 such as the sequence encoding the mature protein is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector.

Recombinant baculovirus is generated by co-transfecting the above plasmid and BaculoGold™ virus DNA (Pharmingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711) using lipofectin (commercially available from GIBCO-BRL). After 4 - 5 days of incubation at 28°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression are performed as described by O'Reilley et al., Baculovirus expression vectors: A Laboratory Manual, Oxford: Oxford University Press (1994).

Expressed poly-his tagged PRO1344 can then be purified, for example, by Ni^{2+}-chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Rupert et al., Nature, 362:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 mL Hepes, pH 7.9; 12.5 mM $MgCl_2$; 0.1 mM EDTA; 10% glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50 mM phosphate, 300 mM NaCl, 10% glycerol, pH 7.8) and filtered through a 0.45 μm filter. A Ni^{2+}-NTA agarose column (commercially available from Qiagen) is prepared with a bed volume of 5 mL, washed with 25 mL of water and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at 0.5 mL per minute. The column is washed to baseline A_{280} with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50 mM phosphate; 300

mM NaCl, 10% glycerol, pH 6.0), which elutes nonspecifically bound protein. After reaching A_{280} baseline again, the column is developed with a 0 to 500 mM Imidazole gradient in the secondary wash buffer. One mL fractions are collected and analyzed by SDS-PAGE and silver staining or Western blot with Ni^{2+}-NTA-conjugated to alkaline phosphatase (Qiagen). Fractions containing the eluted His_{10}-tagged PRO1344 are pooled and dialyzed against loading buffer.

Alternatively, purification of the IgG tagged (or Fc tagged) PRO1344 can be performed using known chromatography techniques, including for instance, Protein A or protein G column chromatography.

EXAMPLE 7

Preparation of Antibodies that Bind PRO1344

This example illustrates preparation of monoclonal antibodies which can specifically bind PRO1344.

Techniques for producing the monoclonal antibodies are known in the art and are described, for instance, in Goding, supra. Immunogens that may be employed include purified PRO1344, fusion proteins containing PRO1344, and cells expressing recombinant PRO1344 on the cell surface. Selection of the immunogen can be made by the skilled artisan without undue experimentation.

Mice, such as Balb/c, are immunized with the PRO1344 immunogen emulsified in complete Freund's adjuvant and injected subcutaneously or intraperitoneally in an amount from 1-100 micrograms. Alternatively, the immunogen is emulsified in MPL-TDM adjuvant (Ribi Immunochemical Research, Hamilton, MT) and injected into the animal's hind foot pads. The immunized mice are then boosted 10 to 12 days later with additional immunogen emulsified in the selected adjuvant. Thereafter, for several weeks, the mice may also be boosted with additional immunization injections. Serum samples may be periodically obtained from the mice by retro-orbital bleeding for testing in ELISA assays to detect anti-PRO1344 antibodies.

After a suitable antibody titer has been detected, the animals "positive" for antibodies can be injected with a final intravenous injection of PRO1344. Three to four days later, the mice are sacrificed and the spleen cells are harvested. The spleen cells are then fused (using 35% polyethylene glycol) to a selected murine myeloma cell line such as P3X63AgU.1, available from ATCC, No. CRL 1597. The fusions generate hybridoma cells which can then

be plated in 96 well tissue culture plates containing HAT (hypoxanthine, aminopterin, and thymidine) medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

The hybridoma cells will be screened in an ELISA for reactivity against PRO1344. Determination of "positive" hybridoma cells secreting the desired monoclonal antibodies against PRO1344 is within the skill in the art.

The positive hybridoma cells can be injected intraperitoneally into syngeneic Balb/c mice to produce ascites containing the anti-PRO1344 monoclonal antibodies. Alternatively, the hybridoma cells can be grown in tissue culture flasks or roller bottles. Purification of the monoclonal antibodies produced in the ascites can be accomplished using ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can be employed.

Deposit of Material

The following materials have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, USA (ATCC):

Material	ATCC Dep. No.	Deposit Date
DNA58723-1588		August 18, 1998

This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the

materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the construct deposited, since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. Isolated nucleic acid comprising DNA having at least an 80% sequence identity to (a) a DNA molecule encoding a PRO1344 polypeptide having the sequence of amino acid residues from 1 or about 24 to 720 of Figure 2 (SEQ ID NO:2), or (b) the complement of the DNA molecule of (a).

2. The isolated nucleic acid molecule of Claim 1 comprising the sequence of nucleotide positions from about 26 or about 95 to about 2185 of Figure 1 (SEQ ID NO:1).

3. The isolated nucleic acid molecule of Claim 1 comprising the sequence of Figure 1 (SEQ ID NO:1).

4. An isolated nucleic acid molecule encoding a PRO1344 polypeptide, comprising DNA hybridizing to the complement of the nucleic acid having the sequence of nucleotide positions from about 26 or about 95 to about 2185 of Figure 1 (SEQ ID NO:1).

5. An isolated nucleic acid molecule comprising DNA having at least an 80% sequence identity to (a) a DNA molecule encoding the same mature polypeptide encoded by the human protein cDNA in ATCC Deposit No. _____ (DNA58723-1588), or (b) the complement of the DNA molecule of (a).

6. The isolated nucleic acid molecule of Claim 5 comprising DNA encoding the same mature polypeptide encoded by the human protein cDNA in ATCC Deposit No. ____ ___ (DNA58723-1588).

7. An isolated nucleic acid molecule comprising (a) DNA encoding a polypeptide having at least an 80% sequence identity to the sequence of amino acid residues from about 1 or about 24 to about 720 of Figure 2 (SEQ ID NO:2), or (b) the complement of the DNA of (a).

8. The isolated nucleic acid molecule of Claim 7 comprising (a) DNA encoding a polypeptide having the sequence of amino acid residues from 1 or about 24 to about 720 of Figure 2 (SEQ ID NO:2), or (b) the complement of the DNA of (a).

9. An isolated nucleic acid molecule comprising (a) DNA encoding a polypeptide scoring at least 80% positives when compared to the sequence of amino acid residues from 1 or about 24 to about 720 of Figure 2 (SEQ ID NO:2), or (b) the complement of the DNA of (a).

10. An isolated nucleic acid molecule having at least 10 nucleotides and produced by hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a PRO1344 polypeptide having the sequence of amino acid residues from 1 or about 24 to about 720 of Figure 2 (SEQ ID NO:2), or (b) the complement of the DNA molecule of (a), and, if the test DNA molecule has at least about an 80 % sequence identity to (a) or (b), isolating the test DNA molecule.

11. A vector comprising the nucleic acid of Claim 1

12. The vector of Claim 11 operably linked to control sequences recognized by a host cell transformed with the vector.

13. A host cell comprising the vector of Claim 12.

14. The host cell of Claim 13, wherein said cell is a CHO cell.

15. The host cell of Claim 13, wherein said cell is an *E. coli*.

16. The host cell of Claim 13, wherein said cell is a yeast cell.

17. A process for producing a PRO1344 polypeptide comprising culturing the host cell of Claim 12 under conditions suitable for expression of said PRO1344 polypeptide and recovering said PRO1344 polypeptide from the cell culture.

18. An isolated PRO1344 polypeptide encoded by the DNA of Claim 1.

19. An isolated PRO1344 polypeptide comprising a polypeptide having at least an 80% sequence identity to the sequence of amino acid residues from 1 or about 24 to about 720 of Figure 2 (SEQ ID NO:2).

20. The isolated polypeptide of Claim 19 comprising amino acid residues from 1 or about 24 to about 720 of Figure 2 (SEQ ID NO:2).

21. An isolated PRO1344 polypeptide scoring at least 80% positives when compared to the sequence of amino acid residues from 1 or about 24 to about 720 of Figure 2 (SEQ ID NO:2).

22. An isolated PRO1344 polypeptide comprising the sequence of amino acid residues from 1 or about 24 to about 720 of Figure 2 (SEQ ID NO:2), or a fragment thereof sufficient to provide a binding site for an anti-PRO1344 antibody.

23. An isolated PRO1344 polypeptide encoded by the cDNA insert of the vector deposited as ATCC Deposit No. _____ (DNA58723-1588).

24. An isolated polypeptide produced by (i) hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a PRO1344 polypeptide having the sequence of amino acid residues from 1 or about 24 to about 720 of Figure 2 (SEQ ID NO:2), or (b) the complement of the DNA molecule of (a), and, if said test DNA molecule has at least about an 80% sequence identity to (a) or (b), (ii) cultuing a host cell comprising said test DNA molecule under conditions suitable for the expression of said polypeptide, and (iii) recovering said polypeptide from the cell culture.

25. A chimeric molecule comprising a PRO1344 polypeptide fused to a heterologous amino acid sequence.

26. The chimeric molecule of Claim 25, wherein said heterologous amino acid sequence is an epitope tag sequence.

27. The chimeric molecule of Claim 25, wherein said heterologous amino acid sequence is a Fc region of an immunoglobulin.

28. An antibody which specifically binds to a PRO1344 polypeptide.

29. The antibody of Claim 28, wherein said antibody is a monoclonal antibody.

30. The antibody of Claim 28, wherein said antibody is a humanized antibody.

Abstract of the Disclosure

The present invention is directed to novel polypeptides having homology to the factor C protein and to nucleic acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention.

FIGURE 1

CGCTCGGGCACCAGCCGCGGCAAGG
><MET {trans=1-s, dir=f, res=1}
ATGGAGCTGGGTTGCTGGACGCAGTTGGGGCTCACTTTTCTTCAGCTCCTTCTCATCTCG
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ATGTGTCGGGAGTGCTGTGAATATGATCAGATTGAGTGCGTCTGCCCCGGAAAGAGGGAA
GTCGTGGGTTATACCATCCCTTGCTGCAGGAATGAGGAGAATGAGTGTGACTCCTGCCTG
ATCCACCCAGGTTGTACCATCTTTGAAAACTGCAAGAGCTGCCGAAATGGCTCATGGGGG
GGTACCTTGGATGACTTCTATGTGAAGGGGTTCTACTGTGCAGAGTGCCGAGCAGGCTGG
TACGGAGGAGACTGCATGCGATGTGGCCAGGTTCTGCGAGCCCCAAAGGGTCAGATTTTG
TTGGAAAGCTATCCCCTAAATGCTCACTGTGAATGGACCATTCATGCTAAACCTGGGTTT
GTCATCCAACTAAGATTTGTCATGTTGAGTCTGGAGTTTGACTACATGTGCCAGTATGAC
TATGTTGAGGTTCGTGATGGAGACAACCGCGATGGCCAGATCATCAAGCGTGTCTGTGGC
AACGAGCGGCCAGCTCCTATCCAGAGCATAGGATCCTCACTCCACGTCCTCTTCCACTCC
GATGGCTCCAAGAATTTTGACGGTTTCCATGCCATTTATGAGGAGATCACAGCATGCTCC
TCATCCCTTGTTTCCATGACGGCACGTGCGTCCTTGACAAGGCTGGATCTTACAAGTGT
GCCTGCTTGGCAGGCTATACTGGGCAGCGCTGTGAAAATCTCCTTGAAGAAAGAAACTGC
TCAGACCCTGGGGGCCCAGTCAATGGGTACCAGAAAATAACAGGGGGCCCTGGGCTTATC
AACGGACGCCATGCTAAAATTGGCACCGTGGTGTCTTTCTTTTGTAACAACTCCTATGTT
CTTAGTGGCAATGAGAAAAGAACTTGCCAGCAGAATGGAGAGTGGTCAGGGAAACAGCCC
ATCTGCATAAAAGCCTGCCGAGAACCAAAGATTTCAGACCTGGTGAGAAGGAGAGTTCTT
CCGATGCAGGTTCAGTCAAGGGAGACACCATTACACCAGCTATACTCAGCGGCCTTCAGC
AAGCAGAAACTGCAGAGTGCCCCTACCAAGAAGCCAGCCCTTCCCTTTGGAGATCTGCCC
ATGGGATACCAACATCTGCATACCCAGCTCCAGTATGAGTGCATCTCACCCTTCTACCGC
CGCCTGGGCAGCAGCAGGAGGACATGTCTGAGGACTGGGAAGTGGAGTGGGCGGGCACCA
TCCTGCATCCCTATCTGCGGGAAAATTGAGAACATCACTGCTCCAAAGACCCAAGGGTTG
CGCTGGCCGTGGCAGGCAGCCATCTACAGGAGGACCAGCGGGGTGCATGACGGCAGCCTA
CACAAGGGAGCGTGGTTCCTAGTCTGCAGCGGTGCCCTGGTGAATGAGCGCACTGTGGTG
GTGGCTGCCCACTGTGTTACTGACCTGGGGAAGGTCACCATGATCAAGACAGCAGACCTG
AAAGTTGTTTTGGGGAAATTCTACCGGGATGATGACCGGGATGAGAAGACCATCCAGAGC
CTACAGATTTCTGCTATCATTCTGCATCCCAACTATGACCCCATCCTGCTTGATGCTGAC
ATCGCCATCCTGAAGCTCCTAGACAAGGCCCGTATCAGCACCCGAGTCCAGCCCATCTGC
CTCGCTGCCAGTCGGGATCTCAGCACTTCCTTCCAGGAGTCCCACATCACTGTGGCTGGC
TGGAATGTCCTGGCAGACGTGAGGAGCCCTGGCTACGAAGAACGACACACTGCGCTCTGGG
GTGGTCAGTGTGGTGGACTCGCTGCTGTGTGAGGAGCAGCATGAGGACCATGGCATCCCA
GTGAGTGTCACTGATAACATGTTCTGTGCCAGCTGGGAACCCACTGCCCCTTCTGATATC
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CCACGCTGGCATCTGATGGGACTGGTCAGCTGGAGCTATGATGAAAACATGCAGCCACAGG
CTCTCCACTGCCTTCACCAAGGTGCTGCCTTTTAAAGACTGGATTGAAAGAAATATGAAA
TGAACCATGCTCATGCACTCCTTGAGAAGTGTTTCTGTATATCCGTCTGTACGTGTGTCA
TTGCGTGAAGCAGTGTGGGCCTGAAGTGTGATTTGGCCTGTGAACTTGGCTGTGCCAGGG
CTTCTGACTTCAGGGACAAAACTCAGTGAAGGGTGAGTAGACCTCCATTGCTGGTAGGCT
GATGCCGCGTCCACTACTAGGACAGCCAATTGGAAGATGCCAGGGCTTGCAAGAAGTAAG
TTTCTTCAAAGAAGACCATATACAAAACCTCTCCACTCCACTGACCTGGTGGTCTTCCCC
AACTTTCAGTTATACGAATGCCATCAGCTTGACCAGGGAAGATCTGGGCTTCATGAGGCC
CCTTTTGAGGCTCTCAAGTTCTAGAGAGCTGCCTGTGGGACAGCCCAGGGCAGCAGAGCT
GGGATGTGGTGCATGCCTTTGTGTACATGGCCACAGTACAGTCTGGTCCTTTTCCTTCCC
CATCTCTTGTACACATTTTAATAAAATAAGGGTTGGCTTCTGAACTACAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
A

FIGURE 2

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></usr/seqdb2/sst/DNA/Dnaseqs.min/ss.DNA58723
><subunit 1 of 1, 720 aa, 1 stop
><MW: 80199, pI: 7.77, NX(S/T): 5
MELGCWTQLGLTFLQLLLISSLPREYTVINEACPGAEWNIMCRECCEYDQIECVCPGKRE
VVGYTIPCCRNEENECDSCLIHPGCTIFENCKSCRNGSWGGTLDDFYVKGFYCAECRAGW
YGGDCMRCGQVLRAPKGQILLESYPLNAHCEWTIHAKPGFVIQLRFVMLSLEFDYMCQYD
YVEVRDGDNRDGQIIKRVCGNERPAPIQSIGSSLHVLFHSDGSKNFDGFHAIYEEITACS
SSPCFHDGTCVLDKAGSYKCACLAGYTGQRCENLLEERNCSDPGGPVNGYQKITGGPGLI
NGRHAKIGTVVSFFCNNSYVLSGNEKRTCQQNGEWSGKQPICIKACREPKISDLVRRRVL
PMQVQSRETPLHQLYSAAFSKQKLQSAPTKKPALPFGDLPMGYQHLHTQLQYECISPFYR
RLGSSRRTCLRTGKWSGRAPSCIPICGKIENITAPKTQGLRWPWQAAIYRRTSGVHDGSL
HKGAWFLVCSGALVNERTVVVAAHCVTDLGKVTMIKTADLKVVLGKFYRDDDRDEKTIQS
LQISAIILHPNYDPILLDADIAILKLLDKARISTRVQPICLAASRDLSTSFQESHITVAG
WNVLADVRSPGFKNDTLRSGVVSVVDSLLCEEQHEDHGIPVSVTDNMFCASWEPTAPSDI
CTAETGGIAAVSFPGRASPEPRWHLMGLVSWSYDKTCSHRLSTAFTKVLPFKDWIERNMK
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Important features of the protein:

Signal peptide:
1-23 MELGCWTQLGLTFLQLLLISSLP

Transmembrane domain:
none

EGF-like domain cysteine pattern signature.
260 CACLAGYTGQRC

N-glycosylation site.
96 NGSW
279 NCSD
316 NNSY
451 NITA
614 NDTL

Serine proteases, trypsin family
489 CSGALVNERTVVvAAHC

CUB domain proteins profile
150 CeWTIhAKPGFVIQLrF

FIGURE 3

GGAGTTTGACTACATGTGCCAGTATGACTATGTTGAGGTTCGTGATGGAG
ACAACCGCGATGGCCAGATCATCAAGCGTGTCTGTGGCAACGAGCGGCCA
GCTCCTATCCAGAGCATAGGATCCTCACTCCACGTCCTCTTCCACTCCGA
TGGCTCCAAGAATTTTGACGGTTTCCATGCCATTTATGAGGAGATCACAG
CATGCTCCTCATCCCCTTGTTTCCATGACGGCAGTGCGTCCTTGACAAGG
CTGGATCTT